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# NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 12 JANUARY 1954 No. 1

## EARLY EXPERIENCES WITH ASCORBIC ACID—A RETROSPECT

Professor Henry C. Sherman, with whom I had the privilege of a post-doctorate year of study in 1926–1927 while on leave from the University of Pittsburgh, had specialized in developing biologic assays that would furnish quantitative measurements of the vitamins recognized at that time. His own early training had included precision work in calorimetry, organic analysis, and mineral balance studies with human subjects. Chemical measurements were impossible then because no vitamin had been identified.

The Columbia University group had succeeded in developing measurements of vitamin A, vitamin $B_1$ and vitamin C, with an estimated accuracy of $\pm 10$ per cent. Little quantitative work had been done on vitamin D, and vitamin $B_2$ represented the growth-stimulating residue in yeast and wheat after autoclaving.

My purpose in spending the year at Columbia was primarily to gain first-hand experience in nutrition and biochemical research with experimental animals as a basis for building up graduate work in that area of teaching and research in the Chemistry Department at the University of Pittsburgh. Research on the molecular structure of fats had been initiated, but my experience with animals had been limited to the boyhood care of farm animals, interspersed with treasured hours of hunting and fishing. However, A. P. Mathews' "Physiological Chemistry," (*4th edition, Wood, New York (1925)*) and Graham Lusk's "The Elements of The Science of Nutrition," (*3d edition, W. B. Saunders Co., Philadelphia (1923)*) had given me a sense of direction that was definite and a natural sequence to working my way through school as a laboratory assistant in the Experiment Station at Washington State College.

Discussions with Professor Sherman and others helped focus my interest on vitamin C. S. S. Zilva and his associates had reported on the preparation of concentrates of the vitamin at the Lister Institute in London, and Nicholai Bezssnoff in France had succeeded in preparing active syrupy material in his laboratories. He suggested the presence of three active compounds: a phenol, a reducing sugar, and an organic acid—not suspecting, apparently, that all three types of reactions might be given by the same substance. Despite the evidence that the vitamin was very unstable, there was some encouragement in the fact that it was readily soluble in water and alcohol and could be held for short periods in concentrated solutions. There was clear evidence that the vitamin was essential in human nutrition and that it was widely distributed in plant and animal tissues, while they were living or fresh.

Three graduate students undertook work on the problem under my supervision during the school year 1927–1928. H. L. Sipple and D. P. Grettie worked directly on the problem of concentrating the vitamin from lemon juice, and R. B. McKinnis undertook electrodialysis studies to find whether the vitamin would show characteristic behavior as an anion, cation, or as a neutral molecule. The experiments of McKinnis showed clearly that the vitamin behaved always as an anion with a diffusion rate comparable to glucose. The precipitation behavior in water and alcohol, with lead and other salt solutions, at varying pH values also indicated that the vitamin was an organic acid of low molecular weight.

Probably no one who was in the laboratory at the time will forget the excitement when Donald Grettie obtained his first crop of white crystals from an alcohol solution of

the vitamin. Within a few hours the crystals were nicely identified as ammonium chloride.

The students built all of the animal cages and other equipment. The only refrigeration available until 1935 was a small icebox that was used jointly by undergraduate and graduate students, and the only centrifuge available was shared with undergraduate students in the food analysis laboratory. The animals were housed in a small room sandwiched between two freshman laboratories a half-mile away. Strangely, we never lost a research assay by infection in the guinea pigs—a suggested explanation being that there was adequate fumigation from the laboratories.

For a time, we bought lemons at the market, but soon the cost became prohibitive. At that stage, we were assisted generously by the California Fruit Growers Exchange by means of a credit, without charge, through their local distributor.

Occasionally there appeared in the literature a suggestion that it was futile to attempt isolation of vitamins because they appeared to be activated sensitive materials that lost their biologic functions when changed to conventional crystalline substances. So far as was known, no vitamin had been isolated as a pure material, but many reported isolations had been proved false, because inactive crystalline products had unwittingly carried the essential nutrients. Nevertheless, crystalline nicotinic acid, inositol, and choline rested peacefully in nearly every biochemical stockroom. Carotenes were under scrutiny but were unproved in their relationship to vitamin A.

F. L. Smith, J. L. Svirbely, and W. A. Waugh as graduate students continued the work and reached an antiscorbutic activity in the range of 1 to 3 mg. of solids per day. After the others had completed their theses in the summer of 1931, Waugh continued the work and by early fall had obtained small yields of crystalline material that retained constant activity at a dosage level of approximately 0.5 mg. per day. To get enough material for animal assays, the preparations had to be repeated twice each week for eight weeks, without interruption. In parallel with the feeding tests, the next series of laboratory steps had to be worked out in anticipation of another ten-week period of quantitative animal tests.

Not wholly incidental to the vitamin studies was the fact that Waugh had a teaching schedule of sixteen laboratory hours per week and my own schedule was twenty-two classroom hours per week (inorganic, industrial, food and sanitary and advanced biologic chemistry).

The conclusions that our product was the pure vitamin seemed to be consistent with all the information available. And it appeared to be identical with a "hexuronic acid" isolated by A. Szent-Györgyi at Cambridge University and later by E. C. Kendall at Mayo Clinic. We were working on a manuscript for publication but were shocked to read press releases from the University of Norway, Oslo (where excellent work on vitamin C had been conducted by Holst, Frölich, and others), stating that Ottar Rygh had identified the vitamin as a well-known alkaloid derivative, methylnornarcotine. The press reports included an account of its isolation from four different sources, followed by chemical synthesis from the parent alkaloid, plus an antiscorbutic activity greater than our product.

The claim was grossly at variance with our consistent pattern of evidence gained through more than four years of study, but no scientific publication was available to permit appraisal of Rygh's evidence. Among other things, the proposed formula made it certain that his substance should behave as a cation in strongly acid solution and should not form an anion except in strongly basic solution, whereas our evidence (McKinnis' paper, 1930) had shown clearly that the vitamin behaved as a nonamphoteric, organic acid.

It seemed necessary to delay publication until we could see Rygh's technical report.

Meanwhile a new series of preparations was initiated, taking our crystalline product through a further lead salt precipitation from alcohol (in which the free vitamin was known to be very soluble) and a subsequent recrystallization from butyl alcohol and petroleum ether. The biologic activity of this product was identical with the first, and by the time the tests were finished, we had secured copies of two of Rygh's papers.

From critical reading of the reports, it seemed probable that the only animals truly free from scurvy in his tests were those that had received, for some strange reason, either an extract of sprouted grain or boiled orange juice as a supplement. Hence we concluded that these supplements had furnished to the animals their only source of vitamin C. A summary paper was then submitted to *Science* and a detailed manuscript was prepared for the *Journal of Biological Chemistry*. A paper was also forwarded for the spring meeting of the American Society of Biological Chemists.

There was still some doubt regarding the chemical nature of any vitamin as described in the literature. Both Jansen and Windaus had obtained crystalline $B_1$, but there was continued uncertainty in regard to its composition and biologic activity (*e.g.*, as reported by Peters). Moore had demonstrated that pure carotene could be converted to vitamin A in chickens, but the nature of the relationship was not clear. Vitamin $D_1$ was shown to be a mixture of $D_2$ with inactive materials. Hence I shall never forget the cordial gesture of Professor McCollum, whom I recognized but had never met, when he greeted me at the conclusion of the paper with a warm handshake and remarked, "Young man, I think you have finally given us a pure compound as a vitamin!" His greeting was symbolic of my feeling toward the graduate students who had done most of the work along the trail and especially Waugh whose former chief had urged him not to work on the problem, because "No one ever isolated a vitamin!"

Confirmation of the report followed rapidly from many sources. Two weeks after our manuscript had been sent to *Science* a letter arrived from Svirbely in which he reported that his work in association with Szent-Györgyi (whom he had joined for post-doctorate work in Hungary) was showing that "hexuronic acid" prepared from adrenal glands was protective against scurvy, when fed to guinea pigs at a level of 1 mg. per day. They were preparing a paper for *Nature*, and later for the *Biochemical Journal*. Within the year, E. K. Nelson (U. S. Dept. of Agriculture) repeated our procedure for isolating the vitamin from lemon juice and added the finding that the inactive fraction most difficult to separate from the final crystals was chiefly inositol, later to be identified as a vitamin by Woolley and as a lipotropic factor by McHenry. E. C. Kendall kindly furnished a supply of "hexuronic acid" that he had isolated by a new procedure from adrenal glands, and we found it to be identical in properties and biologic value with the product from lemons. Tillmanns in Germany, who had detected an association between the dye-reducing value of fruit juices and their vitamin C content, succeeded in isolating the product from citrus fruit and confirmed its antiscorbutic role. Vedder reported similar success. Harris, at Cambridge University, confirmed the antiscorbutic role of "hexuronic acid," but Zilva, who had earlier had a sample in his laboratory, was hesitant to reach agreement, apparently because of the antiscorbutic value of dehydroascorbic acid.

Szent-Györgyi and Svirbely then made possible a widespread attack on the structure of the vitamin by preparing and distributing large quantities from peppers. When the structure had been established, largely through the work of Haworth's group at Birmingham, and by Karrer and Reichstein in Zurich, commercial synthesis followed

rapidly and at low cost from glucose, via sorbitol, sorbose, and ketogulonic acid, so that the product became the first pure vitamin to reach the public by large scale industrial synthesis.

Chemical studies of the vitamin in plant, animal and human tissues progressed rapidly after the substance had been identified. A flood of analytic methods appeared quickly in the literature, but the use of 2,6-dichlorophenolindophenol as a reliable titrating agent in acid solution soon superseded other methods. Tillmann's and Harris' contributions on this score were important, but the method devised by Bessey and Waugh in our laboratory was most widely adopted because of its accuracy, reliability and convenience. Glick then developed a micro method that permitted estimation of the ascorbic acid content of each cell, in microtome slices.

Among the most interesting discoveries from these early analytic studies was the finding that the vitamin was distributed according to a remarkably uniform pattern in the respective tissues of nearly all animals, including those that required the vitamin as an essential nutrient (guinea pigs, monkeys and man, unless subjected to depletion) and those that were not subject to scurvy but maintained a continuous tissue synthesis (rats, chickens, cattle, and nearly all animals studied). The relatively high content of the vitamin in glandular tissues such as the pituitary, adrenals and corpus luteum, and in human milk furnished a portent of its growing significance in relation to health and more broadly, in comparative biochemistry and physiology.

CHARLES GLEN KING
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## VITAMIN $B_{12}$ ABSORPTION AFTER GASTRECTOMY

The availability of vitamin $B_{12}$ isotopically labeled with radioactive cobalt has made possible elegant clinical studies on the absorption of this vitamin in human disease states. The observations of A. D. Welch, V. Scharf, R. W. Heinle, and G. C. Meacham using this tracer have been discussed (*see Nutrition Reviews* **10,** *229 (1952)*). These workers found that patients with pernicious anemia excreted 70 to 95 per cent of the labeled cobalt in their feces while ingesting 0.5 micrograms of the labeled vitamin daily. When intrinsic factor was ingested with vitamin $B_{12}$ the excretion fell to 5 to 30 per cent. These findings provided strong confirmatory evidence supporting the view of L. Berk *et al.* (*New Engl. J. Med.* **239,** *911 (1948)*) that intrinsic factor promotes the utilization of vitamin $B_{12}$.

The use of labeled vitamin $B_{12}$ to study the absorption of this vitamin in patients following gastrectomy has now been reported by M. E. Swendseid, J. A. Halsted, and R. L. Libby (*Proc. Soc. Exp. Biol. Med.* **83,** *226 (1953)*). Four patients who had been subjected to total gastrectomy for carcinoma of the stomach were selected for study. No evidence of recurrent disease was present at the time of study although lymph nodes removed at the time of operation contained cancer in one case. The technic of administration of the $Co^{60}$-labeled vitamin $B_{12}$ was that used previously by Welch *et al.* (*loc cit.*). A dose of 0.5 micrograms of the tracer was given by mouth either alone or with 50 ml. of neutralized gastric juice. The feces were then collected for four days and the excretion of radiocobalt was determined. In 3 of the 4 patients the test was repeated. Without gastric juice very little of the vitamin $B_{12}$ was absorbed and the feces contained from 86 to 103 per cent of the administered dose. A striking change was found after the

administration of gastric juice. In four tests the fecal excretion of labeled cobalt fell to less than 18 per cent. In one test the patient developed diarrhea and excreted 64 per cent of the labeled cobalt. The authors confirmed the poor absorption of labeled vitamin $B_{12}$ by patients with pernicious anemia. Eighty-nine to 100 per cent recovery of labeled cobalt in the feces of such patients was found. The feces of normal individuals similarly tested contained only 25 per cent of the administered labeled cobalt.

It is apparent from these studies that intestinal absorption of vitamin $B_{12}$ after total gastrectomy is as poor as in pernicious anemia and is corrected by the concurrent administration of gastric juice. This finding may be interpreted as indicating that the gastric mucosa is almost the sole source of intrinsic factor. In view of the similarity of the absorptive defect in pernicious anemia and in the postgastrectomy state, the comparative rarity of a megaloblastic anemia following gastrectomy requires explanation. Contributing factors leading to the development of pernicious anemia have been suggested by C. C. Ungley (*Brit. J. Nutrition* **6,** *299* (*1952*)). He has postulated that there is an increased production of toxic or inhibitory factors by intestinal bacteria in pernicious anemia. The presence of such compounds might increase the demand for vitamin $B_{12}$ and precipitate anemia. The occurrence of megaloblastic anemia in experimental animals related to abnormal bacterial growth produced by intestinal stricture and blind loops establishes a precedent for such a theory.

Further clinical studies with vitamin $B_{12}$ labeled with radioactive cobalt would seem to be in order before poor absorption of the vitamin can be equated with the development of anemia. Of particular interest would be a study of patients with achlorhydria and no anemia. Many such patients may show impaired vitamin $B_{12}$ absorption. It will be important to determine whether the onset of megaloblastic anemia can be correlated solely on the degree of absorptive failure, or whether additional precipitating factors can be recognized in both spontaneous and postoperative megaloblastic anemia.

## SERUM CHEMISTRY IN KWASHIORKOR

Kwashiorkor is a form of malnutrition in infants and children that has been reported from a number of geographic locations; it appears to be most prevalent in populations where relatively low quality vegetable proteins comprise the source of this nutrient in the diet of infants and children (*Nutrition Reviews* **9,** *129*, *225* (*1951*); **10,** *87*, *261*, *297* (*1952*)).

The disease is generally characterized by a reduction in serum proteins. Other biochemical disturbances that have been less extensively investigated as possibly characteristic of the disease are: disturbances in blood cholesterol, lowered blood alkaline phosphatase levels as an indication of disturbance in liver function, increases in serum pseudocholinesterase, and decreases in serum calcium, phosphorus and chlorides.

Recently an extensive biochemical investigation was reported by some British workers in order to establish more accurately the disturbances associated with uncomplicated kwashiorkor and to follow the effects of treatment of the disease with appropriate diet (R. F. A. Dean and R. Schwartz, *Brit. J. Nutrition* **7,** *131* (*1953*)).

The subjects were South African children chiefly of the Baganda tribe, who subsisted mainly on bulky vegetable foods low in protein, depending on the staple food supply, and it was estimated that the diet probably did not supply more than 10 to 15 g. of protein daily. The staples of the diet con-

sisted of cooked bananas, sweet potatoes, maize flour, or cassava, cooked in water and eaten with a sauce prepared from a local variety of dried beans. Spinach was often cooked with these other ingredients. These bulky foods were estimated to comprise about 95 to 97 per cent of the total diet; the remainder represented small quantities of animal food of good quality with respect to protein.

Only the children who conformed to the clinical description of the disease (stunted growth, edema, querulous disposition and varying degrees of skin lesions) were included in the study (H. C. Trowell, J. N. P. Davies, and R. F. A. Dean, *Brit. Med. J.* **2,** *798 (1952)*). The children were treated with a low fat, high protein diet, which provided 2 to 3 g. of fat and between 60 and 100 g. of cow's milk protein daily. This diet had proven in earlier studies to promote prompt recovery. The duration of the treatment was usually about twenty-eight days.

The average total serum protein was 3.98 g. per cent (range 2.90 to 5.10) as determined on the first two days of treatment. By the eighth day of treatment this had risen to 6.0 g. and by the twenty-first day the normal value of 7.0 g. per cent had been reached. The albumin fraction, initially at 1.51 g. per cent, rose to 3.6 g., which is still below the normal range of 4.4 to 5.5 g. per cent. Although the globulin fraction was initially at the normal level of 2.44 g. per cent, it rose to 3.60 g. during treatment. Thus, even though the final serum proteins were in the normal ranges, they were characterized by a low albumin : globulin ratio. The average ratio finally attained was just above unity, or about half of what is considered normal.

Children with uncomplicated kwashiorkor had low levels of blood urea (average of 16.2 mg. per cent). This rose to a level far in excess of the normal level of 20 to 25 mg. The peak (60 mg. per cent) was reached on the twenty-first day of treatment, after which there was a gradual decline toward normal values.

The initial level of blood cholesterol was remarkably low (87 mg. per cent). It rose to twice this value in eight days of treatment and at twenty-eight days the average was 140 mg. per cent. The ratio of free to total cholesterol was high initially (range, 0.97 to 0.64) and declined to an average of 0.40 by the twenty-eighth day of treatment. This is still considerably above the ratio for normal children (0.2 to 0.3 as given by H. Behrendt, "Diagnostic Tests for Infants and Children," *Interscience Publishers Inc., New York (1949)*).

At the start of treatment the alkaline phosphatase serum values were 11.8 King-Armstrong units. Fifteen to 20 units is considered normal. The serums varied considerably; the initial values, for example, ranged from a low of 4.7 units to a high of 23.1 units. By the eighth day of treatment the average serum phosphatase values had decreased to 9.5 units; thereafter they gradually increased to an average of 18 units by the twenty-eighth day.

Serum chloride values were within the normal range before treatment and did not change with treatment. The initial average was 620 mg. per cent, expressed as sodium chloride, and the final average was 605 mg. per cent.

Serum amylase and esterase determinations were made to ascertain the influence of frank kwashiorkor on digestive enzymes. All but one of 14 children studied had serum amylase values below 50 Somogyi units. These increased to about 100 units during twenty-eight days of treatment. Serum esterase values were likewise low initially (16.8 King-Armstrong units) and increased rapidly to a level of about 40 units with treatment for fifteen days.

The British study is the most extensive, with respect to describing kwashiorkor in biochemical terms, that has thus far been reported. The authors feel that the lack of

dietary protein can be considered responsible for most, if not all, of the biochemical defects that are characteristic of kwashiorkor. Based on experimental results in animals, however, it should be recalled that the production of nutritional edema (one of the manifestations of kwashiorkor) by restriction of dietary protein alone appears extremely difficult or impossible of attainment. The severe dietary limitation of choline and vitamin $B_{12}$ appeared more important for the production of edema in rats and dogs than did protein deficiency (H. D. Alexander and R. W. Engel, *J. Nutrition* **47,** *361* (*1952*); A. E. Schaefer, D. H. Copeland, and W. D. Salmon, *Ibid.* **43,** *201* (*1951*)). The recovery diet employed by the British workers (high milk-protein) would supply vitamin $B_{12}$ as well as a choline precursor in the form of methionine.

## DIETARY INFLUENCES ON THE COMPOSITION OF THE SALIVA

Numerous investigations have been made of nutritional factors which affect the comparatively constant composition of saliva and their subsequent influences on the oral environment. The presence of free amino acids in the saliva of persons on a normal protein diet as well as on a low protein diet has been demonstrated previously by E. R. Kirch, R. G. Kesel, J. F. O'Donnell, and E. C. Wach (*J. Dent. Res.* **26,** *297* (*1947*); **29,** *779* (*1950*)). In later studies, these investigators (*Ibid.* **32,** *57* (*1953*)) reported on the influence of the ingestion of single amino acids on the salivary concentration of that particular amino acid.

Pre-experimental saliva samples were collected from 2 volunteers at various intervals over a twenty-four-hour period and analyzed microbiologically for the particular free amino acid under study. Then 5 g. of the acid was taken orally; the mouth was rinsed very thoroughly with water which was swallowed. After a two-minute interval, the first of the test samples of saliva was collected, and further samples were taken at intervals over a twenty-four-hour period. These were analyzed by identical procedures. The free amino acids studied in this series were: isoleucine, tryptophan, valine, threonine, cystine, lysine, arginine, leucine, histidine, tyrosine, phenylalanine and methionine. The saliva levels of each of these twelve free amino acids were found to be significantly increased in the sample which was collected two minutes after ingestion of the amino acid; however, the concentrations were back to normal levels within thirty to sixty minutes. The greatest rise was observed with arginine, where an increase from 7.5 to 300.0 micrograms per milliliter of saliva was reported for 1 of the 2 subjects. The next largest increments were noted with leucine and lysine. The lowest increases were observed with methionine and cystine. The very thorough rinsing of the mouth after ingestion of a test dose was believed to eliminate any possibility of any portion of the acids not having been swallowed.

These results are of interest for comparison with the findings of S. W. Hier (*J. Biol. Chem.* **171,** *813* (*1947*)). He reported that the plasma values of free amino acids were higher than normal for a period of at least five hours after ingestion, with the peak reached after two to three hours. This observation is strikingly in contrast with the early peak and rapid return to normal values in saliva. Hier also found that the tyrosine level rose in the plasma when phenylalanine was ingested, but the saliva studies showed no comparable increase in the tyrosine level. The ingestion of methionine, which presumably is a source of cystine to the body, did not cause an increase in the plasma cystine level, nor in the saliva level, but followed the general pattern of all amino acids studied.

The levels of four trace elements (copper, cobalt, nickel and molybdenum) were studied by S. Dreizen, H. A. Spies, and T. D. Spies (*J. Dent. Res.* **31,** *137 (1952)*). Since each of these elements influences one or more of the enzyme systems involved in carbohydrate degradation, their salivary concentration could affect acid production in the oral cavity and the growth of an oral strain of *Lactobacillus acidophilus*. Saliva samples were collected from both caries-active and caries-resistant volunteers and both qualitative and quantitative analyses were made thereon. Of the four trace elements, saliva was found to contain copper regularly, cobalt occasionally, and nickel and molybdenum not at all. The copper content greatly exceeded the cobalt and was approximately 25 per cent as great as that in blood. *In vitro* studies demonstrated that at the maximum quantities observed in saliva, neither copper nor cobalt could affect the rate of acid production in the oral cavity or the growth of an oral strain of *L. acidophilus*. They interpreted these data to suggest that the levels of copper and cobalt in saliva were insufficient to influence dental caries activity. Furthermore, there was no correlation between the copper and cobalt levels determined and the caries susceptibility of the subject. Unfortunately, no trials were made to determine if the ingestion of copper and cobalt salts altered their salivary concentrations.

Human saliva is so well buffered that it maintains a relatively constant pH despite the introduction into the mouth of substances containing high concentrations of acids or alkalis. This homeostatic pH condition is promoted by an increase in the buffer capacity of the saliva, which is secreted in response to stimulation as was observed by J. A. Marshall (*Am. J. Physiol.* **36,** *260 (1915)*). He found that the chewing of paraffin as a salivary stimulant caused the elaboration of a saliva whose neutralizing ability was considerably greater than that collected from the same patient in the resting state. S. Sellman (*Acta Odont. Scandinav.* **8,** *244 (1949)*) observed that at physiologic pH levels, the bicarbonates and phosphates are the principal buffers of saliva, with the bicarbonates predominating in paraffin-activated saliva.

S. Dreizen, A. I. Reed, W. Niedermeier, and T. D. Spies (*J. Dent. Res.* **32,** *497 (1953)*) undertook studies to determine whether the sodium and potassium concentrations of saliva were related to its buffer capacity and if this relationship to the buffer capacity was influenced by the rate of flow. Saliva samples were collected from 80 consecutive patients and records were made of the rate of flow, buffer capacity, sodium and potassium levels. Samples of unstimulated and paraffin-stimulated saliva provided by each of another 25 patients were used to establish the differences of buffering capacity and sodium and potassium levels between resting and activated saliva. Their findings confirmed earlier observations that the buffer capacity of stimulated saliva was considerably greater than that of resting saliva in the same person. The increase in buffering power of stimulated saliva ranged from 27 per cent to 400 per cent over that of resting saliva. The magnitude of difference was, in general, related to the rate of flow. In addition, they found that potassium was the predominant cation in the buffer system of resting saliva, whereas sodium predominated in the stimulated. Their observations substantiated R. Heidenhain's (*Pflüger's Arch. f. d. ges. Physiol.* **17,** *1 (1878)*) original observation that the total percentage of inorganic salts in saliva becomes greater as the rate of secretion is increased. Thus, the greater buffering capacity of stimulated saliva is due to the increase of both sodium and bicarbonate ions.

Recent studies have shown that the adrenal cortex exerts a profound influence on the metabolism of sodium and potassium and that the administration of adrenocorti-

cotropic hormone (ACTH) or cortisone to patients with adrenal cortical insufficiency influenced the blood levels of sodium and potassium. T. F. Frawley and G. W. Thorn (*Proc. 2nd Clin. ACTH Conference* **1,** *115 (1951)*) observed a consistent reduction of the sodium:potassium ratio in unstimulated mixed saliva following the administration of ACTH or cortisone to patients with adrenal cortical hypofunction. It thus appeared that the composition of human saliva could be influenced by these hormones; hence, research was undertaken by S. Dreizen, W. Niedermeier, A. I. Reed, and T. D. Spies (*J. Dent. Res.* **31,** *271 (1952)*) to determine if the administration of ACTH or synthetic cortisone to these patients regulated the sodium and potassium content of saliva independent of the rate of flow. Samples of both resting and paraffin-stimulated saliva were collected from 5 patients being treated with ACTH or cortisone for varying degrees of arthritis. No restrictions were placed on the salt intake. The sodium and potassium contents were determined by flame photometry.

The repeated injection of ACTH or cortisone was followed by definite changes in the concentration of sodium in human saliva. The sodium level of resting and stimulated saliva declined within four hours after the beginning of injections in each of the 5 subjects. The potassium level of the saliva was not materially altered by the hormones with the result that there was a decrease in the sodium:potassium ratio. The potassium content greatly exceeded the sodium content in samples of saliva before, during and after treatment with either ACTH or cortisone. In every patient, the potassium content of saliva was found to exceed the normal blood serum level for this element. In contrast, the blood sodium content in each patient was three to fifteen times as great as the salivary sodium. These studies clearly demonstrate that saliva is not an ultrafiltrate of the blood, but the secretory cells of the human salivary glands possess the faculties of selecting and concentrating certain blood constituents in the elaboration of saliva, a process which is under hormonal as well as neural control.

Other examples of the selective secretions of blood-borne compounds by the salivary glands are found in the studies of the antibiotics conducted by I. B. Bender, R. S. Pressman, and S. G. Tashman (*J. Am. Dent. Assn.* **46,** *164 (1953)*; *J. Dent. Res.* **32,** *287, 435 (1953)*). When aureomycin, chloramphenicol, penicillin, or streptomycin was administered, the peak values in the blood were attained at thirty minutes or earlier. For chloramphenicol, penicillin and streptomycin, the peak values in the saliva also were attained at thirty minutes or earlier and had dropped by about 50 per cent of the original level in two hours. However, in the case of aureomycin, the peak was not reached until one hour after administration and remained high until four hours thereafter. Penicillin and streptomycin disappeared from the saliva much faster than from the blood, whereas chloramphenicol persisted in the saliva almost as long as it did in the blood.

These several series of studies on the relation of systemic alterations to the composition of saliva clearly demonstrate (1) the variability which is possible in the composition of saliva even though it is generally considered to be fairly stable in its composition and (2) the selective ability of the salivary glands in the elaboration of saliva both at rest and during stimulation. In addition, there is every reason to suspect that there are variations between individuals which are predicated by the genetic constitution of the individual and by his physical and emotional status. There seems to be little doubt that the saliva has a more fundamental role in the maintenance of healthy conditions within the oral cavity than is currently conceded. It is a healthy sign that investigators in the fields of nutritional and oral research are becoming more concerned with the exploration of

various relationships to the rate of flow and composition of saliva. This area has been one which, to a large extent, has been neglected by both groups of investigators in the past two or three decades. By reason of the slow metabolic activity in the enamel and dentin of the teeth and the continual bathing action of the saliva on the external surfaces of the teeth, there may easily be a number of unrealized potentialities for influencing the teeth through the ever-present saliva.

## VITAMIN $B_6$ DEFICIENCY IN MAN

Presumptive evidence has led to the conclusion that vitamin $B_6$ is essential in human nutrition. However, no totally clear-cut evidence of a syndrome produced simply by consumption of a diet deficient in this group of vitamins has yet been produced. Clinical observations have been described in which symptoms and signs of deficiency disease have failed to respond to treatment with other members of the vitamin B-complex, but were relieved by administration of pyridoxine (T. D. Spies, W. B. Bean, and W. F. Ashe, *J. Am. Med. Assn.* **112,** *2414 (1939)*; S. G. Smith and D. W. Martin, *Proc. Soc. Exp. Biol. Med.* **43,** *660 (1940)*). A somewhat more direct approach was utilized by S. E. Snyderman, R. Carretero, and L. E. Holt (*Fed. Proc.* **9,** *371 (1950)*). The latter investigators fed highly purified diets deficient in vitamin $B_6$ to infants and observed the occurrence of anemia and convulsive seizures. Administration of pyridoxine was followed by cessation and correction of these manifestations. More extensive investigations of the effects of vitamin $B_6$ deficiency have been undertaken by J. F. Mueller and R. W. Vilter and their associates at the University of Cincinnati (*see Nutrition Reviews* **8,** *182 (1950)*; **10,** *323 (1952)*). In these experiments a vitamin $B_6$-deficient diet has been administered to volunteer subjects at a time when they were also given desoxypyridoxine, a structural analogue and competitive inhibitor of the vitamin. The occurrence of seborrhea-like skin lesions and changes in the tongue and buccal mucosa resembling those of pellagra were described. These lesions cleared promptly within two to three days after administration of pyridoxine.

These investigators have recently published a more detailed description of the symptom-complex produced experimentally by the feeding of desoxypyridoxine and of the metabolic changes associated with it (R. W. Vilter *et al.*, *J. Lab. Clin. Med.* **42,** *335 (1953)*). The original papers from this laboratory had included observations on only 8 patients. The investigations have been extended and the recent report describes observations on a group of 50 patients. All of the subjects suffered from one or another of a group of chronic diseases and their ages ranged from 21 to 87 years. Each patient was carefully examined for manifestations of nutritional deficiency disease before experimental observations were undertaken. Thirty were fed a vitamin B-complex-poor diet while 20 received the regular hospital diet. All received desoxypyridoxine for periods of from three to fifty-three weeks. The daily dosage ranged from 50 to 500 mg. There was variation in potency from one batch of desoxypyridoxine to another, presumably because of variation in the proportions of contaminating pyridoxine. This made it difficult to compare activities from one experiment to another.

Thirty-four of the 50 subjects who received desoxypyridoxine developed symptoms and signs considered to be those of vitamin $B_6$ deficiency. Lesions first appeared as early as the fifth day. In other patients administration of desoxypyridoxine for periods as long as a year failed to cause

appearance of signs and symptoms. In general lesions appeared earlier and more frequently in subjects who received the diet deficient in vitamins of the B-complex than in those who were on a regular hospital diet.

The most common manifestation was seborrheic dermatitis occurring usually about the eyes, in the eyebrows, and at the angles of the mouth. Such a lesion occurred in 29 subjects and was the lesion first appearing in 24. Glossitis developed in 14 patients and was the first manifestation in 5. The changes in the tongue could not be distinguished from those occurring in acute niacin deficiency. Similarly, there were occasional manifestations in the mucous membranes of the mouth like those of niacin deficiency. Conjunctivitis, cheilosis and angular stomatitis which were comparable to such lesions in riboflavin deficiency occurred in a few patients.

Three patients developed signs of a severe sensory neuritis with sensations of numbness and tingling in the hands and feet, hyperesthesia, and impairment of vibration and position sense. Tendon reflexes became hypoactive. After treatment with pyridoxine the sensory manifestations abated, but the syndrome of "hot foot" or "electric foot" occurred with manifestations like those previously described in prisoners-of-war in the Pacific area (D. Denny-Brown, *Medicine* **26,** *41* (*1947*)). An erythematous lesion of the skin with distribution and later occurrence of scaling and pigmentation like that in pellagra was observed in 2 patients.

Common subjective symptoms of deficiency included anorexia, nausea, vomiting, lethargy, somnolence and confusion. Six patients developed intercurrent infections during the period of desoxypyridoxine administration. There was little apparent influence of the deficiency on the chronic diseases from which the subjects suffered. Several experiences are worth noting, however. One patient with psoriasis had rapid spread of skin lesions during the deficiency with regression when treatment with pyridoxine was begun. There was a decrease in size of the enlarged lymph nodes and spleen in patients with chronic lymphatic leukemia.

The subjects were treated with one of the members of the vitamin $B_6$-group while the dietary regimen and administration of desoxypyridoxine continued. Pyridoxine, pyridoxal, or pyridoxamine were given either orally or parenterally in doses of 5 to 200 mg. daily. There was no discernible difference in effectiveness of response to treatment among the three members of the group of vitamins and small doses were apparently as effective as larger doses.

The response to treatment was prompt, with disappearance of nausea and improvement in appetite within twelve hours. Burning sensations of the tongue and mucous membranes of the mouth disappeared in twenty-four hours and these structures had returned to normal in forty-eight hours. Skin lesions, cheilosis and angular stomatitis cleared in from three to eight days. Manifestations of neuritis disappeared more slowly and when the "hot foot" syndrome followed therapy it lasted for several months. The authors also record the interesting observation that pyridoxine applied locally in an ointment to skin lesions effected healing at the site of application. If applied over larger areas of skin other clinical manifestations respond as well, indicating absorption and utilization of the vitamin.

Other vitamins were administered to some patients prior to treatment with vitamin $B_6$. It is of significance that large doses of thiamine, niacin, and riboflavin failed to affect dermatitis, glossitis, cheilosis, or neuritis. In 2 patients, however, the daily intravenous administration of from 25 to 50 mg. of diphosphopyridine nucleotide was associated with prompt healing of lesions of glossitis and stomatitis.

The investigators used a variety of laboratory procedures in an effort to char-

acterize the changes in blood constituents and in metabolic systems in the deficiency state induced by desoxypyridoxine. The most consistent change in the formed elements of blood was the occurrence of an absolute decrease in circulating lymphocytes. This was observed in 17 of 18 patients and usually developed at the time deficiency signs first appeared. An increase in eosinophils was found in patients with extensive skin lesions. The lymphopenia and eosinophilia were corrected within seven days of treatment with pyridoxine. Although a mild normocytic normochromic anemia occurred in some patients, it was not possible to ascribe this defect with certainty to the vitamin $B_6$ deficiency.

Tryptophan metabolism is abnormal in vitamin $B_6$ deficiency in experimental animals and in such states an abnormal metabolite, xanthurenic acid, appears in the urine (*Nutrition Reviews* **8,** *36 (1950)*). A test dose of 5 g. of L-tryptophan or 10 g. of D,L-tryptophan was given to 34 of the patients and the quantity of xanthurenic acid excreted in the urine in twenty-four-hour periods immediately preceding and after the administration of the test dose was determined. Thirty of the subjects had a marked increase in the quantity of xanthurenic acid excreted during periods of ingestion of desoxypyridoxine. Usually this abnormality became apparent long before signs or symptoms of deficiency had occurred and was corrected within five days after administration of pyridoxine was begun. Normally no more than 30 mg. of xanthurenic acid is excreted within a twenty-four-hour period after the test dose of tryptophan. The large majority of these patients excreted from 100 to 500 mg. during the period of induction of the deficiency. However, there was no correlation between severity or type of manifestations and the quantity of xanthurenic acid excreted. The quantity of N-methylnicotinamide excreted in the urine of these subjects was not reduced or changed during the deficiency period or after administration of pyridoxine.

There was a tendency for the concentration of urea nitrogen in blood to increase during the period of ingestion of desoxypyridoxine and to decrease when supplements of vitamin $B_6$ were given. These changes were enhanced when urea nitrogen was determined twelve hours after the administration of 12 g. of alanine. Other blood constituents determined were unchanged during the period of deficiency. These included glucose, lipids, total cholesterol and cholesterol esters. Also, there were no abnormalities in the results of intravenous or oral glucose tolerance tests.

A variety of tests of function of the adrenal cortex were undertaken. No evidence was adduced indicating significant changes in adrenocortical function in vitamin $B_6$ deficiency.

The observations described represent a monumental nutritional investigation. The number of subjects included in the study is large enough to afford a comparatively clear picture of the manifestations resulting from use of the antimetabolite. The fact that all symptoms and signs failed to respond to other essential nutrients, yet were relieved by pyridoxine even though administration of desoxypyridoxine was continued, would seem to make wholly valid the conclusion drawn by the authors that the manifestations are in reality those of vitamin $B_6$ deficiency.

Another point of great interest is the variety of signs and symptoms which may occur in vitamin $B_6$ deficiency induced by this means. The chief and most common clinical manifestations were those of anorexia, nausea, lethargy, seborrheic dermatitis, cheilosis, conjunctivitis, glossitis, pellagra-like dermatitis, and polyneuritis. Each of these may occur in man as manifestations of nutritional deficiency of other members of the B-complex group of vitamins, as in thiamine, niacin, or riboflavin deficiency. This finding may account for the delay in

recognizing spontaneously occurring vitamin $B_6$ deficiency in patients. Certainly it has not been an unusual experience in the past to find that certain of these manifestations responded poorly or slowly to administration of thiamine, niacin, or riboflavin. It should be pointed out, also, that the known metabolic roles of vitamin $B_6$ relate it closely to activities of other B-complex vitamins in a variety of metabolic systems.

Laboratory studies indicate that the deficiency is associated with lymphopenia, disruption in normal patterns of tryptophan metabolism so that xanthurenic acid is excreted in the urine, and accumulation of urea in the blood.

The data recorded do not permit deduction of the critical ratio of pyridoxine to desoxypyridoxine in overcoming the effects of the antimetabolite. This occurred because the desoxypyridoxine used was contaminated with variable and unknown quantities of pyridoxine. However, it was determined that as little as 5 mg. of pyridoxine was sufficient to effect healing of deficiency lesions induced by feeding as much as 200 mg. of desoxypyridoxine.

These experiments are admirable in the clarity with which they were planned and the information they afford. They demonstrate clearly that vitamin $B_6$ is required in the diet of man and that the deficiency syndrome is one with manifestations not readily differentiated from those occurring in thiamine, niacin, or riboflavin deficiency.

## ANTIBIOTICS IN AVIAN NUTRITION

Some of the growth stimulation derived from the addition of antibiotics to poultry feeds was suggested to involve the control of transmissible agents which accumulate in an environment where poultry are housed and grown year after year (*Nutrition Reviews* **10,** *270 (1952)*). Recent experimental results with chicks are in support of this view.

D. C. Hill, H. D. Branion, S. J. Slinger, and G. W. Anderson (*Poultry Sci.* **32,** *462 (1953)*) fed diets with and without penicillin to chicks in two animal houses. One of these houses had served as shelter for chicks for many years and the other was a new structure previously unused. Growth response to penicillin was observed to occur earlier and was greater in chicks housed in the old quarters than in the previously unused new quarters.

These workers also noted some differences in the relative numbers of various types of organisms present in the cecum. In general, the over-all viable bacterial population was greater for the birds in the old animal quarters than in the new quarters. The chicks housed in the old quarters and fed penicillin contained fewer cecal enterococci than chicks in the same quarters not receiving penicillin. Chicks housed in the new quarters actually increased their cecal numbers of enterococci in the presence of dietary penicillin. An evaluation of these findings is difficult. However, the authors noted that in general the changes in the cecal flora in chicks housed in new quarters were quite different from the cecal flora in chicks housed in old quarters, not only with respect to enterococci but also with respect to aerobes. The antibiotic consistently resulted in a lowering of the pH of the cecal contents; this observation is in keeping with that reported earlier from the same laboratory (G. W. Anderson, J. D. Cunningham, and S. J. Slinger, *J. Nutrition* **47,** *175 (1952)*).

The influence of environment on the growth response to antibiotics in chickens was also investigated by R. J. Lillie, J. R. Sizemore, and H. R. Bird (*Poultry Sci.* **32,** *466 (1953)*). The rate of growth of chicks fed no antibiotic and housed in new

quarters was observed to be superior to that obtained with aureomycin supplement when the shelter consisted of old quarters in which poultry had been housed previously.

In chicks housed in old quarters the growth-stimulating effect of antibiotics was evident after one week; this effect increased in magnitude until at least the fourth week, and was much greater than was obtained in chicks housed in new quarters. The mild response to antibiotics in chicks kept in the new quarters was not evident until the fourth week in one experiment and the second and third weeks in two other experiments. Chicks grown for the first four weeks in new quarters with antibiotic in their diet suffered a decline in growth when deprived of the antibiotic and moved to old quarters. This decline in growth did not appear when, after four weeks, the antibiotic was withdrawn from chicks that were kept continuously in old quarters.

These results suggest that chicks grown in quarters continuously used for poultry are subjected to a microbial environment that retards growth early. Antibiotics under these conditions afford partial protection and permit more rapid early growth. The results of T. D. Luckey (*Feedstuffs* **24,** *No. 24, p. 10 (1952)*) are in agreement with such an explanation, since under germ-free conditions chicks and turkey poults did not respond to antibiotics. Lillie and co-workers point out that in the past the early retardation of chick growth has been considered normal because of its almost universal occurrence. Whether or not this explanation of antibiotic action as a growth stimulant can be extended to other species has not been established, although a similar explanation was advanced by J. M. Sieburth *et al.* (*Proc. Soc. Exp. Biol. Med.* **76,** *15 (1951)*) for growth-stimulating effects of antibiotics in pigs.

The response of the duckling to antibiotics has been investigated by H. D. Branion, G. W. Anderson, and D. C. Hill (*Poultry Sci.* **32,** *335 (1953)*). Aureomycin hydrochloride, penicillin G potassium, terramycin hydrochloride, and streptomycin sulfate at levels of 25 parts per million (p.p.m.) failed to influence growth rate or efficiency of feed utilization in one experiment. In another experiment the aureomycin, penicillin, and terramycin were tried at three dietary levels: 10, 25, and 100 p.p.m. in the diet. There was again no indication of growth stimulation from these supplements. Even though these antibiotic supplements failed to stimulate growth in the duckling, they were observed to lower the pH of the cecal contents and also to modify the cecal microbial populations.

These authors present an excellent review (ninety-six references to the literature) of the various theories that have been advanced regarding the mode of action of antibiotics as growth stimulants in animals. With regard to the behavior of ducklings, they concluded that this species is not susceptible to the mild unknown infections which affect the growth of chicks and turkey poults under the same environmental conditions. The failure of ducklings to respond to antibiotics is not common to water fowl, since growth response to antibiotics in goslings has been reported from the same laboratory (S. J. Slinger, E. S. Snyder, W. F. Pepper, and G. W. Anderson, *Poultry Sci.* **31,** *941 (1952)*; H. D. Branion and D. C. Hill, *Ibid.* **31,** *1100 (1952)*).

## THE EVALUATION OF NUTRITIONAL DEFICIENCIES IN SHEEP

Significant nutritional problems limiting national sheep production on the typical rough hill grazing terrain which covers over half the area of Scotland have been studied by C. F. Mills (*Chem. Ind. (London), No. 15, 330 (1953)*). This is a particularly serious

problem since the Scottish sheep industry furnishes about one fifth of Scotland's agricultural output. Most of these highland areas have been used solely for sheep grazing with the object of producing an annual crop of lambs for sale to lowland farmers for fattening. The nutritional demand placed upon these grazing areas is graphically exemplified by the calculation that 35 tons of lime are removed from the soil each year in the 3000 square miles of Argyllshire alone. General malnutrition is considered the most serious cause of losses among hill flocks. When this is accompanied by unfavorable weather, the losses may be extremely heavy among lambing ewes, for example, 3,000,000 deaths in 1947, or many lambs may be born dead or die shortly after birth.

New growth of herbage is available during only five months of the year because of climatic conditions. During the other seven months of the year, the sheep are forced to subsist on the remains of the summer grazing, which have become high in lignified tissue and low in available energy and protein. This situation is very serious in relation to the nutritional demands of the breeding ewe. Under hill conditions, mating usually occurs in November so that the period of pregnancy is during the winter months of minimum grazing. During the last four to six weeks of pregnancy when there is a marked increase in the fetal demand for proteins and minerals, the ewe is forced to exist on such a low plane of nutrition that she is losing weight although pregnant.

A. M. Thomson and W. Thomson (*Brit. J. Nutrition* **2,** *90* (*1949*)) have studied the weight changes of ewes on natural hill grazing and report that a characteristic drop in body weight commences in September before mating begins, and a steady weight decrease continues until by the lambing period in April the average weight loss is almost 25 per cent of the total body weight. Body weight losses as high as 50 per cent were not infrequent.

These workers conducted an experiment to study the effect of restricting the dietary intake of the pregnant ewe. One group of ewes was maintained on an adequate plane of nutrition and the other subjected to a dietary restriction half way through pregnancy sufficient to cause a gross weight loss of 5 per cent. Even this milder dietary restriction caused an increased number of stillbirths, an impaired vitality in the lambs at birth, decreased milk production by the ewe and over-all appreciably increased lamb losses. When the ewes had twins, this additional strain resulted in even more drastic losses among those on the restricted diet. For example, 82 per cent of the twins born to ewes on the normal diet survived whereas only 14 per cent of the 44 twins born to ewes on the restricted diet survived.

Economic objections are raised to supplementary feedings, since the artificial feeding of an animal, whose survival in hill areas depends on its ability to forage for itself, must lead to hardship. The effects of changes in management, the introduction of mixed stock and the possibilities of liming and fertilizing accessible hills are all suggested as possible remedies. The nutritional evaluation of such changes has been measured by three methods: blood analyses, chemical analyses of hill herbage, and growth studies of young grazing animals. A. H. H. Fraser, W. Godden, and D. W. Auchinachie (*Biochem. J.* **28,** *157* (*1934*)) have shown that the blood calcium level may give an unreliable index of calcium deficiency in the ewe since blood composition may remain relatively normal even when growth and bone development are retarded. J. Duckworth, Godden, and W. Thomson (*J. Agr. Sci.* **33,** *190* (*1943*)) investigated the effect of varying the vitamin D intake in lambs maintained on a ration containing adequate calcium and phosphorus and showed that serum calcium levels were influenced greatly by variations in energy intake and rate of growth. Rickets did not develop unless there was moderate rate of growth and a serum calcium level below 7 mg. per cent.

When analyses of herbage from hill grazing areas indicated deficiencies in calcium and calories, supplementary feedings were administered to a number of hill sheep flocks to determine which nutrient was necessary or more important in order to achieve greater productivity. However, after a six-year period, A. H. H. Fraser (*Tr. Highland Agr. Soc. (Scotland)* **51,** *39 (1939)*) concluded that the feeding of mineral supplements alone did not improve either the lambing percentage or live weight gains in young stock. When minerals were fed together with supplementary energy-rich foods, marked improvement followed, but the energy supply appeared to be of greater value than the minerals.

Investigations are now under way by Mills (*loc. cit.*) to determine whether fluctuations in the chemical composition of the carcass of the sheep may give a more reliable index of the nutritional adequacy of its grazing than studies of the herbage or blood analyses. Since reproduction and lactation under duress force an animal to draw on its skeletal reserves, these changes in bone composition and structure were studied in ewes maintained on diets containing different quantities of calcium and phosphorus. It was found that certain bones of the skeleton are highly sensitive to demands by the lactating ewe for calcium and phosphorus. The thoracic vertebrae, for example, became heavily depleted of ash constituents if the mineral intake remained suboptimal for a prolonged period. Thus the determination of the total ash content of such selected bones was an index of nutritional adequacy. Radiographs of living or dissected animals showed marked differences in bone structure and porosity between animals maintained on different calcium and phosphorus intakes. Radiographic examination of the tibias differentiated animals maintained on a calcium-deficient diet from those receiving a calcium supplement. Energy intake was found to have a significant effect on the depletion of skeletal reserves during pregnancy and lactation. Analyses of the carcass for minerals, proteins, and fats registered a far more reliable index of the nutritional status of the animal than determination of live weight changes. Further investigations of all these nutritional measurements are under study on three flocks maintained on hill grazings and also on animals at the Rowett Research Institute at Aberdeen, Scotland.

Copper and cobalt are the two trace elements most commonly deficient in the diet of the grazing hill sheep of Scotland. Copper-deficient ewes frequently gave birth to deformed lambs. Diagnosis of this deficiency by blood analyses is of little value, as ewes with low blood copper may also give birth to normal lambs. Because of the apparently normal copper content of herbage in these areas where copper deficiency of livestock is found it would seem that some "conditioning factor" rendered dietary copper non-available to the animal. W. S. Ferguson (*Vet. J.* **104,** *145 (1948)*) reported on studies conducted to determine if high molybdenum or lead intakes might be a causative factor and demonstrated that neither of these elements is involved.

C. F. Mills (*Thesis, University of London (1951)*) has shown that the copper of herbage exists in the form of organic complexes and that a number of water-soluble copper complexes found in herbage of normal sheep are not present in the herbage of areas where sheep show copper deficiency. Thus some organic factor may be causing interference to normal nutrition by "binding" copper in complex formation, or by indirectly affecting the metabolism. Cobalt deficiency shows itself as a wasting disease leading to a severe anemia with a progressively failing appetite. Many outbreaks are directly attributable to low levels of cobalt in the soil inducing a deficiency in the herbage. A. T. Phillipson and R. L. Mitchell (*Brit. J. Nutrition* **6,** *176 (1952)*) studied the site of action of cobalt in sheep, and have found that intravenous injection of cobalt salts will neither prevent nor cure the deficiency. Their results sup-

ported the hypothesis that cobalt exerted its beneficial action within the rumen, reticulum, or omasum. They found that liver cobalt stores did not give a physiologic measure of cobalt adequacy and suggested that the analyses of rumen contents gave a more reliable index of the adequacy of cobalt intake. In areas of deficiencies the best methods of prevention were to give animals access to cobalt-containing mineral supplements or to sprinkle the grazings with a small quantity of cobalt sulfate.

These nutritional problems, although especially acute in the highlands of Scotland, undoubtedly apply in lesser degree to other grazing areas of the world. The appraisal of these nutritional deficiencies and the devising of methods which are economically feasible for the correction of these problems have been discussed in some detail above. In terms of food and wool production in the British Isles alone, the solution of the present situation could readily provide large increases in these two commodities which would enable the British people to be more readily self-sufficient in these staples. In an era of rapidly expanding world population the correction of such devastating losses in an area which is otherwise unproductive would be a worthwhile contribution to the nutriture and economic status of mankind.

## METABOLISM OF UREA

Urea has long been considered an end product of protein metabolism in mammals. This would appear to be substantiated, at least insofar as urea nitrogen is concerned, by the observations of A. San Pietro and D. Rittenberg (*J. Biol. Chem.* **201,** *445 (1953)*) that the urea space in man as measured by the dilution of a tracer quantity of $N^{15}$-labeled urea given intravenously is quantitatively the same as the total body water space as determined by the dilution of injected deuterium oxide ($D_2O$). However, evidence that urea carbon may take part in further reactions, *in vivo*, has been obtained by two groups of workers.

E. Leifer, L. J. Roth, and L. H. Hempelmann (*Science* **108,** *748 (1948)*) injected 0.5 mg. of $C^{14}$-labeled urea intraperitoneally into 9 mice of the $CF_1$ strain. The $C^{14}$ content of the urine, expired air, and various tissues was measured at several intervals, up to forty-eight hours, after the injection. No mention is made of the food and water intake of the animals during this period. Radioactivity was detected in the urine and expired air very soon after the injection of the tagged urea. A total of 21 per cent of the injected $C^{14}$ was found in the expired air during the forty-eight hours of collection. The rate of expiration of the $C^{14}$ was greatest in the first few hours, about 15 per cent being exhaled in six hours. During the same period of time, 84 per cent of the injected $C^{14}$ was found in the urine, 35 per cent of which was excreted in the first three hours following injection. All the radioactivity in the urine was in the *urea* fraction. The biologic half-life of the tagged urea was five hours.

At various time intervals after injection of the tagged urea, animals were killed and certain tissues were examined for radioactivity. No details of these measurements are given. Presumably, the $C^{14}$ content of the various tissue fractions was measured together. The $C^{14}$ urea was uniformly distributed on a weight basis within 25 per cent in the blood, brain, muscle, heart, liver and spleen. The $C^{14}$ content of the kidneys was two to three times that of the other tissues tested. Leifer and his coworkers ascribe this to the concentrating action of the renal tubules.

S. H. Zbarsky and W. D. Wright (*Canad. J. Med. Sci.* **31,** *151 (1953)*) have reported a similar study in rats. In one of their three

experiments, a 250 g. male albino rat, presumably on a normal diet, was injected intraperitoneally with 0.5 ml. of a 0.9 per cent saline solution containing 10 mg. of $C^{14}$-labeled urea. The rat was immediately placed in a closed metabolism apparatus. Food and water were offered ad libitum. Expired air and urine were collected at intervals for forty-eight hours, at the end of which time the animals were anesthetized (type of anesthesia is not stated), heparinized, and bled as completely as possible from the carotid artery. The liver, kidney, spleen, stomach and intestines were removed and combined. These organs were dried, extracted with an alcohol-ether mixture, and pulverized. Their $C^{14}$ content was measured either by direct counting of the tissue powder or after its combustion to carbon dioxide. The remainder of the carcass was dissolved in hot concentrated potassium hydroxide and the radioactivity of the resultant solution was measured.

Radioactive carbon appeared in the expired air carbon dioxide shortly after the injection of the tagged urea. In the first hour, about 4 per cent of the injected $C^{14}$ was exhaled, in the second hour 6 per cent, and in the third 5 per cent. By the twelfth hour about 27 per cent of the injected $C^{14}$ had appeared in the expired air. Relatively little was thereafter exhaled; the total exhaled in forty-eight hours was about 30 per cent. The rapid falling off of $C^{14}$ in the expired air is presumably secondary to the rapid urinary excretion of the tagged urea. The specific activity of the excreted urea was high during the first few hours. About 35 per cent of the injected $C^{14}$ was excreted in the first six hours, about 60 per cent in twelve hours, and about 63 per cent in forty-eight hours. As in the study of Leifer *et al.*, all the excreted $C^{14}$ was in the *urea* fraction. Approximately 50 per cent of the injected $C^{14}$ was lost in the expired air and urine in five hours. This value of five hours for the biologic half-life of the injected $C^{14}$-labeled urea is the same as that observed in mice by Leifer *et al.*

Zbarsky and Wright found no radioactivity in the alcohol-ether tissue extracts. Minimal activity was found in the tissue powder (combined liver, kidney, spleen, etc.), while about 5 per cent was found in the carcass. No attempt was made to determine in which nitrogen fraction this activity occurred.

In order to study the distribution of radioactivity in various tissues before most of the $C^{14}$ had been lost in the urine and expired, a rat was killed three hours after the injection of a similar quantity of tagged urea. About 9 per cent of the injected $C^{14}$ appeared in the expired carbon dioxide and 32 per cent in the urine during this time. One per cent was found in the liver urea, 1.3 per cent in the blood and kidney urea, and 0.03 per cent in the blood carbon dioxide. Only traces of radioactivity were found in the proteins of the tissues examined or in fat extracted from the stomach and intestines. Forty-five per cent of the injected radioactivity was found in the carcass. The nature of the radioactive material found in the carcass was not determined, but the authors feel it was probably in the form of urea.

The high specific activity of the urea excreted in the urine during the first few hours after injection of the $C^{14}$-labeled urea suggests that a considerable fraction of the injected urea was excreted without entering into any metabolic reaction. Further, there was little incorporation of the $C^{14}$ in the tissue fat or proteins; the radioactivity found in the tissues was essentially all in the urea fraction. In contrast to these data, which are compatible with the idea of urea being an end-product of protein metabolism, is the rapid appearance of a considerable proportion of the radioactivity of the injected $C^{14}$-labeled urea in the expired air carbon dioxide. This is interpreted by the two groups of investigators to indicate that urea undergoes considerable metabolic activity.

The nature of the conversion of the carbon of urea to carbon dioxide in the

expired air is not known. The data regarding $N^{15}$-labeled urea and the lack of incorporation of $C^{14}$ from $C^{14}$-tagged urea in tissue proteins or fat suggest that perhaps an exchange reaction is occurring. It should be mentioned that in both the studies of Leifer *et al.* and those of Zbarsky and Wright, quantities of tagged urea in excess of what might be considered tracer doses were employed.

## INFLUENCE OF VITAMIN $B_{12}$ AND ALPHA-TOCOPHEROL ON LIVER VITAMIN A STORAGE

Studies on the role of vitamin $B_{12}$ in metabolism have centered largely around its relationship to the synthesis of methyl groups and its relationship to nutrients supplying methyl groups, as reported by A. E. Schaefer, W. D. Salmon, and D. R. Strength (*Proc. Soc. Exp. Biol. Med.* **71,** *202 (1949)*; *Nutrition Reviews* **9,** *231 (1951)*; **10,** *27, 285 (1952)*; **11,** *220 (1953)*).

Recently, evidence has been presented to indicate that vitamin $B_{12}$ improves the efficiency of utilization of dietary carotene for liver storage of vitamin A in rats (E. G. High and S. S. Wilson, *J. Nutrition* **50,** *203 (1953)*). The study was prompted by the suggestion of E. C. Callison, L. F. Hollman, W. F. Martin, and E. Orent-Keiles (*Arch. Biochem. Biophys.* **32,** *407 (1951)*) regarding the desirability of including vitamin $B_{12}$ or liver extract in vitamin A bioassay diets.

Rats deficient in both vitamin A and vitamin $B_{12}$ were divided into equal groups by sex and growth rate and supplemented daily for twenty-one days with 33 micrograms of carotene dissolved in 0.2 ml. of cottonseed oil. This supplement was fed on a small amount of basal diet which was usually consumed in thirty minutes. Additional treatment consisted of daily intramuscular injections of 0.2 ml. of saline solution (group 1, controls), 0.2 ml. of saline solution containing 0.28 micrograms of vitamin $B_{12}$ in the form of liver extract (group 2), or crystalline vitamin $B_{12}$ (groups 3 and 4). The animals were killed sixteen hours after the last injection and their livers were analyzed for vitamin A. The livers of the control animals contained 46.4 micrograms of vitamin A, whereas those of treatment groups 2, 3 and 4 (vitamin $B_{12}$-supplemented) contained 65.0, 61.1, and 64.6 micrograms, respectively (averages of 5 to 8 animals per treatment).

In order to inhibit microbial synthesis of vitamin $B_{12}$, the experiment was repeated and 2 g. of sulfasuxidine were added to the basal diet during depletion and also during the supplementation period. Under these conditions, a 33 microgram intake of carotene daily resulted in a storage of 36.6 micrograms of vitamin A per liver, contrasted with 57.6 and 56.3 micrograms when vitamin $B_{12}$ was injected with carotene. A comparable increase in vitamin A storage in the liver was obtained when the vitamin $B_{12}$ was included in the ration as an animal protein factor (APF) supplement (55 micrograms of vitamin $B_{12}$ per kilogram of diet).

These results indicated that vitamin $B_{12}$ increased the utilization of carotene for tissue deposition of vitamin A. To determine whether similar influences would be operative if preformed vitamin A was supplied to the animals, 13 micrograms of vitamin A were administered to depleted rats in the presence and absence of vitamin $B_{12}$. In this case there was no indication of a definite vitamin $B_{12}$ effect on liver vitamin A storage; the average liver storage of vitamin A ranged between 32.4 and 39.3 micrograms per liver in controls and between 33.7 and 41.1 micrograms per liver in vitamin $B_{12}$-supplemented animals. These results are suggestive of a possible function of vitamin $B_{12}$ in the enzymatic conversion of carotene to vitamin A or in improving the efficiency of carotene absorption.

In their studies High and Wilson used the

USP vitamin A bioassay diet which consists of the following, in per cent: vitamin A-free casein, 18; glucose, 58; salts, 4; dried brewer's yeast, 8; irradiated yeast, 2; and hydrogenated cottonseed oil, 10. These workers, however, did not supplement the diet with tocopherols which are known to increase the utilization of carotene when added to somewhat similar diets (*Nutrition Reviews* **9,** *217 (1951)*; **10,** *149 (1952)*).

The results of High and Wilson are of particular interest in view of the apparent similarity of behavior of vitamin $B_{12}$ to that of tocopherols as observed by others in improving carotene utilization. In this connection it is noteworthy that vitamin E and vitamin $B_{12}$ can partially replace each other in promoting protein utilization and in protecting rats against acute carbon tetrachloride toxicity (E. L. Hove and J. O. Hardin, *Proc. Soc. Exp. Biol. Med.* **77,** *502 (1951)*).

The apparent role of tocopherols in improving carotene utilization was further investigated by J. W. Hebert and A. F. Morgan (*J. Nutrition* **50,** *175 (1953)*). These workers noted that the addition of 0.5 mg. of alpha-tocopherol daily to the diet of partially vitamin A-depleted rats receiving 35 to 129 micrograms of vitamin A daily produced no significant change in the liver stores of vitamin A. Similar tocopherol supplements in partially depleted rats receiving 87 to 174 micrograms of carotene in oil daily produced a significant increase in liver stores of vitamin A. Above and below these levels of carotene intake, tocopherols had no influence on vitamin A storage. These workers also made the important observation that carotene in aqueous solution (Tween 40) produced larger amounts of liver vitamin A storage than carotene administered in an oily medium. Tocopherol, under these conditions, did not increase liver storage of vitamin A but actually seemed to have a depressant effect.

The inability of tocopherols to improve liver vitamin A stores derived from dietary carotene when the latter are administered in aqueous mediums suggests that the tocopherols as well as vitamin $B_{12}$ may exert their beneficial effects upon carotene utilization through an improvement of absorption from the digestive tract. This is in line with the earlier reported observations of others that vitamin $B_{12}$ exerts a real and marked influence in favor of improved energy metabolism (*Nutrition Reviews* **10,** *308 (1952)*).

With regard to vitamin $B_{12}$ and its relationship to the utilization of methyl sources (choline, methionine, etc.), it is noteworthy that choline deficiency impairs the utilization of both carotene and vitamin A for hepatic deposition of the latter (H. Popper and H. Chinn, *Proc. Soc. Exp. Biol. Med.* **49,** *202 (1942)*).

The results of High and Wilson (*loc. cit.*) suggest that in the bioassay of carotene, the assay diet fortified with vitamin $B_{12}$ would probably represent a truer picture of carotene value as a vitamin A source under practical conditions than similar diets low in or devoid of vitamin $B_{12}$.

## PANTOTHENIC ACID AND THE PITUITARY-ADRENAL AXIS

In a brief review of some current aspects of pantothenic acid in nutrition by W. A. Krehl (*Nutrition Reviews* **11,** *225 (1953)*), evidence is summarized which seemingly shows an insufficiency of adrenal cortical function in pantothenic acid-deficient animals. The evidence involves a lack of eosinopenia and lymphocytopenia in response to epinephrine administration, increased insulin sensitivity and a diminished ability to synthesize hepatic glycogen during fasting. These defects were reported to be corrected by cortisone administration but not by the injection of ACTH.

An investigation of the effects of pantothenic acid deficiency on the pituitary-adrenal system of the rat has been reported by B. H. Ershoff, R. B. A. Slater, and J. G. Gaines (*J. Nutrition* **50,** *299* (*1953*)). The results here are at variance to some extent with those reported from other laboratories. In experiment 1, 86 weanling female rats of the Long-Evans strain were divided into three groups, one to receive the pantothenic acid-free ration ad libitum, a second to receive the complete diet ad libitum and a third to receive the complete diet, but to be pair-fed with the deficient animals. After thirty days on these diets, the animals received a single injection of epinephrine or of ACTH. Total and differential white cell counts were made immediately before injection and two, four and twenty-four hours afterwards. Injection of epinephrine into control animals receiving the complete ration ad libitum resulted in a marked decrease in lymphocyte count at two and four hours after injection. Similar treatment of pantothenic acid-deficient rats led to an increase in lymphocytes at two hours after injection, but there was a significant decrease at four and twenty-four hours after injection. The pair-fed controls showed a moderate decrease in lymphocyte count two hours after injection, with a return to normal at four hours. The injection of ACTH led to lymphocytopenia in all three experimental groups. The decrease was smallest in the pair-fed controls. The ready response to the administration of ACTH suggests that the adrenals are functioning normally in the pantothenic acid-deficient animal. The failure of epinephrine to elicit a cellular response two hours after injection while being effective four hours after injection suggests that the pituitary secretion of ACTH is delayed.

In experiment 2 the ascorbic acid and cholesterol contents of the adrenals were determined. These two compounds are frequently employed to indicate the functional capability of the adrenal. A total of 160 female rats of the Wistar strain (found by the authors to have more uniform adrenal weights and cholesterol levels than the Long-Evans rats) were placed on the three dietary regimens employed in experiment 1, that is, pantothenic acid-deficient ration, complete ration fed ad libitum and the complete ration pair-fed with the deficient animals. After twenty-four days on these regimens, the rats were injected with ACTH or epinephrine, anesthetized later with nembutal and killed by drawing blood by cardiac puncture. The adrenals were weighed and analyzed for ascorbic acid and cholesterol. Hemorrhagic adrenals were not subjected to chemical analysis. The animals fed the complete ration ad libitum responded to both ACTH and epinephrine administrations with marked reductions in adrenal ascorbic acid and cholesterol. In panthothenic acid-deficient rats the administration of ACTH or epinephrine reduced the adrenal content of ascorbic acid. The cholesterol level, however, was not changed. While the ascorbic acid results indicate normally functioning adrenals and pituitary, the cholesterol results suggest a failure of the adrenals to respond to ACTH. The authors point out, however, that the cholesterol content of pantothenic acid-deficient adrenals is much lower than that of normal animals, and that it may be impossible for the cholesterol level to be depressed still further. In pair-fed control rats ACTH and epinephrine reduced the ascorbic acid content of the adrenals. Epinephrine also reduced the cholesterol content, while ACTH was ineffective. These results are difficult to interpret.

A third criterion employed by the authors to test the functioning of the pituitary-adrenal axis is that of egg white toxicity. Parenteral administration of diluted egg white is fatal to adrenalectomized rats but not to rats with intact adrenals or to adrenalectomized rats treated with cortisone. Pantothenic acid-deficient rats survived the egg white treatment, as did control animals.

This suggests that they are capable of producing at least minimal amounts of cortical hormones.

In view of the considerable variability of the data, statistical treatment would have been helpful. Clearly, much more work is required to establish reliable criteria of pituitary-adrenal function and to reconcile the divergent results reported from different laboratories.

## FATTY LIVERS IN AMINO ACID DEFICIENCIES

The production of fatty livers on low protein, high fat diets (C. H. Best, J. M. Hershey, and M. E. Hunstman, *J. Physiol.* **75,** *56 (1932)*) opened a field of investigation which has proved highly fruitful. Best and Huntsman (*Ibid.* **75,** *405 (1932)*) later showed that when choline was added to the diet, hepatic lipid infiltration was prevented. A. W. Beeston, H. J. Channon, and H. Wilkinson (*Biochem. J.* **29,** *2659 (1935)*) observed that in the absence of choline, high levels of casein showed a preventive effect. H. F. Tucker and H. C. Eckstein (*J. Biol. Chem.* **121,** *479 (1937)*) demonstrated that the lipotropic agent in these protein supplements was methionine.

More recently H. C. Eckstein (*J. Biol. Chem.* **195,** *167 (1952)*; *Nutrition Reviews* **10,** *187 (1952)*) endeavored to demonstrate that methionine alone, among amino acids, possesses lipotropic properties. His method consisted in comparing liver fat levels obtained with four diets, one containing 5 per cent casein, one 35 per cent casein and two containing a 9.53 per cent amino acid supplement with 0.2 and 1.2 per cent methionine added, respectively. Fatty livers were produced on the low casein and low methionine diets. In a second experiment each of the essential amino acids was added to diets containing 5 per cent casein plus cystine. The levels of amino acids corresponded to those found in the 35 per cent casein ration. Only methionine reduced the liver fat to the level observed with the 35 per cent casein control ration. It appeared, therefore, that among the essential amino acids only methionine exerted a lipotropic action in the liver.

However well established this conclusion seemed to be, it has been successfully challenged by a recent report of S. A. Singal, S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn (*J. Biol. Chem.* **200,** *867 (1953)*). These workers found that on a low (9 per cent) casein diet, even when a low fat ration was used and despite the presence of choline, fatty livers developed. Addition of a mixture of threonine, valine and histidine prevented hepatic lipid infiltration. Of these three amino acids, threonine proved to be the effective agent. Phenylalanine, tryptophan, nicotinic acid, cobalamin and folinic acid proved to be of no value. Neither was serine of value, thus showing that the effect of threonine was not solely due to its hydroxyl group. Threonine was found to be ineffective when choline was entirely eliminated from the diet. Conversely, large supplements of choline and methionine had some lipotropic effect on low threonine diets. These results suggested to the authors that there are two separate yet related phenomena in fatty liver production, first, a primary deficiency of methyl group or choline in which threonine is without effect and second, primary threonine deficiency in which choline is effective at high dietary levels.

Repeating these experiments with amino acid mixtures replacing the "basal" casein, Singal and his collaborators observed that the complete exclusion of threonine from the ration caused complete growth failure but no alteration in liver lipids. Supplements of 0.4 to 0.7 per cent of threonine resulted in improved growth rates and, simultaneously, increased liver lipids. (The 0.7

per cent level corresponds to the threonine content of 9 per cent casein.) Further increase in the threonine content improved growth to near optimal rates and reduced liver lipids to normal.

Similar results were obtained when the lysine content of the diet was varied. No growth and no significant fatty infiltration were observed on lysine-free diets. Graded supplements of lysine from 0.35 to 0.57 per cent resulted in improved growth and in considerable fatty infiltration. Higher levels resulted in near optimal growth and in normal liver lipids. The production of "lysine fatty liver" as well as of "threonine fatty liver" of a maximal fat content was obtained when growth took place at 80 per cent of the optimal rate, with the requirements for growth apparently taking precedence over the requirements for the maintenance of normal liver lipids.

The composition of lysine and threonine fatty livers resembled that found in choline deficiency. With the increase in total liver lipids there was a decrease in liver phospholipids and iodine number of the total lipids. In addition, appreciable amounts of esterified cholesterol were found. Serum cholesterol (free and total) and phospholipids did not differ from those observed in animals on adequate rations.

In a second study S. A. Singal, S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn (*J. Biol. Chem.* **200,** *875* (*1953*)), further investigated the effect of threonine deficiency on the synthesis of phospholipids and phosphoproteins. Their aim was to elucidate the previously discovered relationship between this amino acid and choline. They found that threonine deficiency depressed the synthesis of the phospholipids and of the pentose nucleic acid fractions of liver. Supplementary threonine stimulated their turnover to near normal values within eight hours. These changes were not observed in the small intestine or in the kidney. Threonine supplement had no effect in rats fed an adequate ration. The fact that the response to threonine was obtained within a lapse of time during which no consistent lipotropism was demonstrable suggests that a failure of fatty acid oxidation (resulting from impaired microsome function) is the cause of the fatty infiltration. In choline deficiency impairment of fat transport is the primary physiologic lesion.

Finally, S. A. Singal, S. J. Hazan, V. P. Sydenstricker, and J. M. Littlejohn (*J. Biol. Chem.* **200,** *883* (*1953*)) demonstrated that of the various related compounds D-, L-, and DL-threonine, DL-allothreonine and DL-homoserine, only L-threonine prevented fatty livers and stimulated growth on the deficient diet.

The results of these workers may well have important clinical significance. Massive fatty infiltration of the liver is the chief internal symptom of kwashiorkor (*Nutrition Reviews* **4,** *52*, *303* (*1946*); **6,** *270* (*1948*); **8,** *55*, *161* (*1950*); **10,** *261* (*1952*)) and is observed frequently in African babies showing no visible sign of malignant malnutrition (*Ibid.* **10,** *297* (*1952*)). Partial "subdeficiencies" of these two amino acids may well be of importance in human nutrition as they are known to be present in limited amounts only in many staple vegetable proteins.

## *IN VITRO* STUDIES OF VITAMIN A FORMATION

The site of conversion of carotene to vitamin A in the animal body has been discussed previously (*Nutrition Reviews* **7,** *135* (*1949*)). The older view was that the liver was the principal organ which split carotene to form vitamin A. More recent evidence has suggested that the intestine is the organ primarily concerned. A direct

approach to this problem was made by C. E. Wiese, J. W. Mehl, and H. J. Deuel (*Arch. Biochem.* **15,** *75* (*1947*)), who studied the conversion of carotene to vitamin A. The appearance of vitamin A was recognized by the production of a blue color when antimony trichloride was added to the nonsaponifiable lipid extract. An average of 2.6 micrograms of vitamin A was apparently formed in one hour from carotene by the isolated intestine of the rat.

Confirmation of the above result has been sought by A. Rosenberg and A. E. Sobel (*Arch. Biochem. Biophys.* **44,** *320* (*1953*)). The specificity of the color reaction previously employed was questioned and better methods were devised. In their work, Rosenberg and Sobel detected the presence of vitamin A by the difference in the absorption spectrum of the nonsaponifiable lipid before and after irradiation with ultraviolet light, as suggested by A. Chevallier and P. Chabre (*Bull. Soc. chim. biol.* **16,** *1451* (*1934*)).

Experiments were performed using the technic of Wiese *et al.* (*loc. cit.*). An aqueous carotene dispersion was administered by stomach tube to vitamin A-depleted rats. The rats were killed immediately and the upper and lower ends of the intestine were ligated. Incubation of the intestinal loop was carried out for one hour in a Ringer's solution. The contents of the intestine were flushed out and the intestinal wall was analyzed for vitamin A. The absorption of light at 328 millimicrons was measured before and after irradiation with ultraviolet light. An average synthesis of 4.24 micrograms of vitamin A was found.

In a subsequent paper A. Rosenberg and A. E. Sobel (*Arch. Biochem. Biophys.* **44,** *326* (*1953*)) have compared the conversion of carotene to vitamin A by the intestine of normal and alloxan-diabetic rats. The experimental procedure was that described above. The intestine from nondiabetic rats produced 4.24 micrograms of vitamin A, while the *in vitro* production of vitamin A by the intestine from alloxan-diabetic rats was only 0.76 micrograms. The authors have calculated from their *in vitro* experiments that the normal rat could produce 51 micrograms of vitamin A in thirty-six hours, whereas the alloxan-diabetic rats could produce only 8.5 micrograms. These calculations agree remarkably well with the measured *in vitro* increase in stored vitamin A of 40 micrograms in normal rats and 10 micrograms in diabetic rats thirty-six hours after carotene was provided (A. E. Sobel, A. Rosenberg, and H. Adelson, *Ibid.* **44,** *176* (*1953*)).

These studies provide further direct experimental evidence that the intestine, at least in the rat, is very important in the conversion of carotene to vitamin A. Technically the measurement of vitamin A by the spectrophotometric difference method is subject to much less criticism than earlier technics. The rat differs from the human being in that carotene is not absorbed from the intestine. In man some carotene probably can be absorbed from the intestine. It would seem that the excessive rise of blood carotene in diabetic patients following oral carotene administration may be attributed to improved carotene conversion. In the rat the intestine seems to be almost the exclusive organ for conversion of carotene. The degree to which the human liver may participate in vitamin A formation has not been established.

## THE *LACTOBACILLUS BIFIDUS* FACTOR

Studies made during the last quarter-century have greatly increased our knowledge of the composition of both human and cow's milk. The biochemical differences between the milks from these two species have been the center of much controversy

concerning their merits in the feeding of human infants. Modifications of cow's milk mixtures rather closely simulating human milk have been developed and extensively used. However, milk is one of the more complex of natural foods and it is not surprising that differences among the milks of various species continue to be found.

A hitherto unrecognized difference between human milk and cow's milk has been reported by P. György (*Pediatrics* **11,** *98 (1953)*). During the course of a study of the nutritional requirements of *Lactobacillus bifidus* under anaerobic conditions in a chemically defined medium, György and his associates noted the appearance of a mutant which required a growth factor found in relatively high concentrations in human milk, especially in the colostrum. This factor was also found in a lesser concentration in a wide variety of human and animal secretions. Except for the colostrum, cow's milk was found to be a poor source of this factor. It could not be replaced by any of a large number of substances frequently stimulatory to the growth of microorganisms.

The observation that the blood group substances, meconium, gastric mucin, and polysaccharides from certain types of pneumococci possessed a high degree of activity suggested that this new factor might be mucopolysaccharide in nature. D-glucosamine, L-fucose, and D-galactose were identified following acid hydrolysis of concentrates of the factor. However, blood group activity was not present in all of the active preparations from human sources. This variant of *L. bifidus* apparently produces an enzyme which reduces the serologic blood group activity of meconium concurrently with the liberation of dialyzable reducing substances, chiefly acetylglucosamine. The growth-promoting activity of these concentrates for *L. bifidus* is destroyed under similar conditions.

The occurrence in mother's milk of a potent growth factor for a variant of one of the most prevalent organisms found in the intestine of the breast-fed human infant naturally leads to much speculation concerning its significance. The conclusion that the presence of the *L. bifidus* factor in human milk is responsible for the development of the bacterial flora typifying the nursing infant is all too attractive. The fact that many strains of *L. bifidus* isolated by these workers did not require this factor would suggest caution in such an interpretation. However, it seems likely that a basic biochemical observation has been made. Further studies on the nature of this factor will be awaited with interest.

## DIETARY ATHEROSCLEROSIS IN MONKEYS

The study of experimental atherosclerosis has been limited largely to a small number of animal species. The greatest amount of work has been done on rabbits and chickens. Because these species differ greatly from man in their diet and general metabolism, the production of experimental atherosclerosis in primates is a worthwhile objective. Feeding rhesus monkeys a high cholesterol diet for as long as ten months has been found unsuccessful in producing atheromata (R. Kawamura, "Neue Beiträge zur Morphologie der Cholesterinsteatose," *p. 267, Gustav Fischer, Jena (1927)*). Vascular lesions have however been reported in rhesus monkeys fed a diet deficient in pyridoxine for six months (J. F. Rinehart and L. D. Greenberg, *Am. J. Path.* **25,** *481 (1949)*). The lesions produced were plaques of intimal fibrosis unassociated with excessive lipid deposition. These observations have not as yet been confirmed.

The successful production of atherosclerosis in the Cebus monkey by dietary

means has been described by G. V. Mann, S. B. Andrus, A. McNally, and F. J. Stare (*J. Exp. Med.* **98,** *195 (1953)*). The addition of 0.5, 1.0 and 2.0 per cent cholesterol to a purified diet containing casein as the protein source produced only slight rises in the serum cholesterol even after as long as twenty-six weeks of feeding. No greater rise occurred in the serum cholesterol of alloxan-diabetic monkeys as compared to normal monkeys fed the casein diet supplemented with 1 or 2 per cent cholesterol. A very different result was obtained when cholesterol was added to a diet low in available methionine. This diet, devised for another purpose, had the alpha protein of soybean meal as a protein source. Alpha protein has been shown to be low in available methionine (C. R. Grau and M. Kamei, *J. Nutrition* **41,** *89 (1950)*). Monkeys fed this diet became anorectic, lost weight and developed fatty livers unless choline was added.

When 5 per cent cholesterol was incorporated into the choline-containing alpha protein diet the plasma cholesterol rose from the average normal level of 142 mg. per cent to levels in excess of 300 mg. per cent in most cases. Methionine added to the alpha protein diet prevented the rise in cholesterol levels. The hypercholesterolemia, once established by the experimental diet, could also be cured by the addition of 1 g. of DL-methionine daily to the diet. It was found that 0.5 g. of L-cysteine was only partially effective in lowering the level of blood cholesterol.

The changes in the serum lipoproteins of monkeys produced by the experimental diets have been measured. The normal flotation pattern of the Cebus monkey is quite similar to that of man except that there is practically no lipoprotein with a flotation rate exceeding $S_f30$. When the monkeys were fed the diets which produced hypercholesterolemia, changes in the normal lipoprotein pattern occurred. The first change was an increase in the $S_f3$ to 8 material, followed by increases in the $S_f8$ to 12 and $S_f12$ to 20 classes. Very little additional material was found in the larger lipoproteins, $S_f20$ to 100. There was little increase in the serum neutral fat or phospholipid and in most cases the serum remained clear.

Seventeen monkeys fed the alpha protein diet supplemented with cholesterol and choline have been examined pathologically. These animals maintained a serum cholesterol over 300 mg. per cent during the experimental period. Of the 9 monkeys which were hypercholesterolemic for eighteen or more weeks, marked atheromata in the aortas of 4 were noted. Four monkeys had minimal lesions and one monkey was free of aortic disease. Lesions were less consistently found in monkeys fed the experimental diet for shorter periods.

The lesions themselves were smooth, noncalcified, pale yellow plaques which reached 1 cm. in diameter. Most of the plaques were found in the ascending portion and arch of the aorta. The orifices of the larger arteries were frequently involved. Lesser involvement of the major arteries, including the coronary arteries, was occasionally found. The aortic and mitral valves were also the site of atheromatous changes. In microscopic sections the lesions were found to be composed of "foam cells" with a small number of lymphocytes, fibroblasts and polymorphonuclear leukocytes. Clefts of cholesterol crystals were noted in some plaques. No changes were seen in the internal elastic lamina. Histologic evidence suggested that the lesions contained free and esterified cholesterol in addition to neutral fat. Examination of the livers showed that only an occasional monkey demonstrated abnormal lipid deposits. This is to be contrasted with the marked visceral cholesterolosis which occurs in rabbits after cholesterol feeding.

The contribution of Mann and co-workers to the study of the influence of diet on atherosclerosis is an important one. From a metabolic point of view the dependence on a relative methionine deficiency in the production of hypercholesterolemia suggests a

new role for the sulfur-containing amino acids. Despite the difficulty of prolonged dietary studies on monkeys, further use of this species should give information more applicable to human atherosclerosis than that obtained from subprimate species.

## RELATIVE RATES OF INTESTINAL ABSORPTION OF D- AND L-AMINO ACIDS

Comparison of the metabolic activity and availability of the stereoisomers of the amino acids has interested many investigators (*Nutrition Reviews* **4,** *173 (1946)*; **8,** *202, 230 (1950)*; **10,** *241 (1952)*). Observations recorded by Q. H. Gibson and G. Wiseman (*Biochem. J.* **48,** *426 (1951)*; *Nutrition Reviews* **9,** *343 (1953)*) indicated that the L-isomer disappeared from intestinal loops more rapidly than did the corresponding D-form. These experiments were carried out in loops of ileum in the surviving rat and did not rule out the possibility of metabolic breakdown within the intestine.

Other evidence of an active and selective process of absorption in the intestine has been sought by W. T. Agar, F. J. R. Hird, and G. S. Sidhu (*J. Physiol.* **121,** *255 (1953)*). The procedure used was to determine rates of transfer against a concentration gradient in an isolated loop of intestine *in vitro*. A specimen of about 40 cm. of small intestine was mounted in an apparatus so that two circuits of circulating fluid might be maintained, one within the lumen and the other bathing the serosal surface. Temperature, volume, and hydrostatic pressure of the fluid were kept constant. In both circuits Krebs' bicarbonate solution gassed with 5 per cent carbon dioxide in oxygen and containing glucose in a concentration of 500 mg. per cent was utilized. Various amino acids were added to the fluid in the inner circuit or to both fluids. Experiments were continued for one or two hours and the concentration of amino acid in each of the fluid circuits was determined at intervals.

Under the conditions established in these experiments there was passage of water from the inner to the outer circuits with an average rate of 0.17 ml. per centimeter of intestine per hour. It was observed, also, that glucose was transferred at a rate faster than water, indicating active transfer of this substance across the intestinal wall. Moreover, not all of the glucose disappearing from the fluid in the lumen could be accounted for in the outer fluid. This quantity was undoubtedly metabolized by the tissue.

Amino acids were added to both fluids in a concentration of 30 micromoles per milliliter at the beginning of experiments. The quantity of amino acid appearing in the outer circuit must be understood to be dependent on the quantity of water passing the barrier and the specific processes of amino acid transport. Both factors were taken into account. By such means it was demonstrated that L-histidine moved across the intestinal wall at a rate greatly exceeding that of the D-isomer of this amino acid. Since the rate of transfer of D-histidine was less than that of water, it was apparent that the L-isomer moved against a concentration gradient. Similarly, L-phenylalanine was transferred actively across the intestinal barrier, while the D-isomer of this amino acid was transferred even more slowly than water. In contrast, L-glutamic acid was not transferred actively against the concentration gradient.

A variable quantity of amino acid in the experiments with phenylalanine and histidine could not be accounted for by shifts from the inner to outer circuits of fluid. Analysis of the intestine for the specific amino acid provided evidence that the gut wall took up quantities comparable to those unaccounted for in the circulating fluids. It was also found that the tissue was first saturated with the amino acid before any appeared in the outer fluid.

The effect of enzyme inhibitors was tested on this system. Cyanide reduced the rate of transfer of water and glucose and totally blocked the active transfer of L-histidine. Similarly, dinitrophenol reduced the rate of transfer of L-histidine by 75 per cent.

The intestinal absorption and metabolism of certain peptides were also studied. It was determined that these were largely hydrolyzed within the lumen, but small quantities of dipeptide were found to pass the intestinal barrier into the outer circuit of fluid. The peptides used in these experiments were glycylglycine, glycylglycylglycine, and L-leucylglycine.

The results indicate that the L-isomers of certain amino acids, in these experiments L-histidine and L-phenylalanine, are absorbed by an active process of transfer since their movement is against a concentration gradient. The D-isomers, in contrast, are apparently absorbed passively through processes of simple diffusion. Moreover, the more rapid transfer of L-isomers is inhibited by cyanide and dinitrophenol. The findings provide an explanation of the physiologic mechanisms responsible for the more rapid disappearance of L-isomers than D-forms of amino acids from the intestinal tract which had been observed by earlier investigators.

## FAT ABSORPTION IN ABSENCE OF BILE AND PANCREATIC JUICE

Despite the many studies of fat absorption, its mechanism is not completely known. The markedly diminished absorption of fat in patients with biliary and pancreatic disease called attention early to the importance of the liver and pancreas in this process. However, the specific modes of action of bile and pancreatic juice in fat digestion and absorption have not been definitely established. To gain more information about this, V. C. Pessoa, K. S. Kim, and A. C. Ivy (*Am. J. Physiol.* **174,** *209* (*1953*)) have recently compared the absorption of neutral fats and free fatty acids in normal, biliary-fistula, and pancreatic duct-ligated dogs. The adequacy of each biliary fistula and pancreatic duct ligation was proved at the end of each experiment at autopsy.

The basal diet for the dogs was composed of casein (vitamin-free), corn syrup, cellulose powder and a salt mixture. Corn oil and a mixture of fatty acids, obtained by hydrolysis of corn oil, were used as the dietary fat in the majority of studies. When fat or fatty acids were added to the diet, the total daily caloric and protein intakes (grams) were kept the same as in the fat-free diet by adjusting the proportion of calories from carbohydrates and proteins. The daily protein intake (grams) was kept constant because of the reported effects of protein on bile acid output (F. F. Coburn and J. Annegers, *Am. J. Physiol.* **163,** *48* (*1950*)) and fecal fat excretion (D. F. Magee, K. S. Kim, and A. C. Ivy, *Ibid.* **167,** *807* (*1951*)). The daily caloric intake for each dog was kept constant throughout the study. Approximately 80 calories per kilogram of body weight per day were given to dogs weighing more than 8 kg., and 100 calories per kilogram of body weight per day to dogs weighing less than 8 kg.

Each dog received one multivitamin tablet every few days. Despite the addition of methionine (amount not stated) to the daily diet of the pancreatic duct-ligated dogs, all these animals were found (at autopsy) to have developed fatty livers.

The endogenous lipid fecal excretions measured while the dogs were on the fat-free diets were about 40, 140, and 125 mg. per kilogram per day for normal, biliary fistula and pancreatic duct-ligated dogs respectively. The difference between each type of operated dog and the normal was statistically significant; the difference between the biliary fistula and pancreatic duct-ligated dogs was not significant.

The concept that the intestinal tract is the primary source of the endogenous fecal fat is supported by the observation that while the thoracic duct lymph of normal dogs fasted for several days contains considerable fat (about 400 mg. per kilogram per day), very little fat is found in the chyle of fasting enterectomized dogs. Synthesis of fat by the intestine, mobilization of intestinal fat, and absorption of fat that has been excreted into the intestine are possible sources of the fat found in the intestinal lymph of fasting dogs. There is little evidence to suggest synthesis of fat by the intestinal bacteria. Neither the bile nor pancreatic juice appear to be necessary sources of the endogenous fecal fat, since the endogenous fecal fat excretion is greater than normal in the absence of either.

Pessoa and co-workers suggest that desquamating intestinal mucosal cells are the principal source of endogenously excreted fat. This is based in part on the observation of C. P. Leblond and C. E. Stevens (*Anat. Record* **100,** *357 (1948)*) that the life span of rat intestinal mucosal cells is about one and one-half days. This means that about two thirds of the intestinal mucosa is desquamated daily. If a similar situation exists in the dog, the amount of fat which would be available from desquamating mucosal cells is sufficient to account quantitatively for the endogenous fecal fat excretion since it has been calculated by W. M. Sperry and R. W. Angevine (*J. Biol. Chem.* **96,** *769 (1932)*) that the total fat content of the intestinal mucosa per kilogram for the dog is about 250 mg. In normal dogs presumably most of the lipids excreted into the gut are reabsorbed, but in the absence of biliary or pancreatic excretions a high proportion presumably is not absorbed, thereby accounting for an increased endogenous fecal fat excretion in these animals.

When corn oil was added to the basal diet, the absorption of the ingested fat was almost complete (an average of 97 per cent) in the normal dogs. In contrast, significantly less absorption (70 to 75 per cent) occurred in both the biliary fistula and pancreatic duct-ligated dogs. No significant difference was observed between the types of operated dogs. Impaired fat absorption in the biliary fistula and pancreatic duct-ligated dogs was also noted when either oleic acid, a commercial dog food containing tallow fat as its principal fat, or tallow fat was eaten. When corn-oil fatty acids were ingested, the greatest average absorption (89 per cent) was again noted in the normal dogs; an average of 71 per cent was absorbed in the pancreatic duct-ligated animals and 55 per cent in the biliary fistula dogs. Additional data are required to establish the significance of the apparent lesser absorption of fatty acids than of fat in the normal and biliary fistula dogs.

To gain information on the degree of hydrolysis of fats occurring in the intestinal lumen, Pessoa and co-workers measured the percentage of soap in the fecal fat of a small number of dogs. They found that the fecal soap : total fat ratio varied with the different diets and with the type of animal. In normal dogs on a fat-free diet, 25 to 70 per cent of the endogenous fecal fat was present as soaps. A similar percentage was observed when corn oil or corn-oil fatty acids were fed to normal dogs. A somewhat lesser proportion of the fecal fat was present as soap in dogs with biliary fistulas on the fat-free or corn-oil diets. However, when such dogs ingested corn-oil fatty acids or tallow fat, the fecal fat soap percentage was in the "normal" range. In dogs with pancreatic duct ligations, the fecal soap percentage was low when the dog ingested the fat-free, corn-oil fatty acids, or corn oil diets. They suggest that the relatively small amount of soap found in the pancreatic duct-ligated dogs ingesting corn-oil fatty acids may be due to an insufficient supply of alkali for saponification in the absence of pancreatic juice. No measurements of available alkali were made, however.

No soap was found in the fecal fat in two

dogs with pancreatic duct ligations receiving corn oil. This is interpreted by the authors to indicate that minimal hydrolysis of fat had occurred in the intestines of these animals. Since about 70 per cent of the ingested corn oil was absorbed, Pessoa *et al.* consider this crucial evidence against complete lipolysis as a requisite for fat absorption.

When tallow fat was ingested by dogs with pancreatic duct ligations, the fecal soap:fat ratio was only slightly lower than in normal dogs ingesting corn oil. No data are presented on normal dogs ingesting tallow fat.

These data concerning fecal soap excretion suggest that the biliary and pancreatic secretions (particularly the latter) are necessary for the hydrolysis of fats composed of unsaturated fats (corn oil), but not for the hydrolysis of fats containing chiefly saturated fatty acids (tallow fat). The latter hydrolysis can apparently be accomplished by the enzymes of the succus entericus. Since soap is found in the endogenous fecal fat of dogs with pancreatic duct ligations, Pessoa *et al.* infer that the lipids excreted with the intestinal lumen contain saturated fatty acids.

Fecal phospholipid excretion was also measured in a small number of dogs. All but one of the normal dogs excreted only minimal amounts of lipid phosphorus in their feces on all the diets tested. One normal dog excreted a large amount of fecal fat phosphorus while on the corn-oil fatty acid diet. No explanation for this excretion was apparent. While only traces of fecal lipid phosphorus were excreted by the biliary fistula dogs on the fat-free diet, the phospholipid excretion increased abnormally when these dogs ingested corn oil or corn-oil fatty acids. Much less of a rise was observed when tallow fat was eaten. No data are available on normal dogs ingesting tallow fat. The fecal lipid phosphorus excretions of the dogs with pancreatic duct ligations on the various diets were low, as in the normal dogs. As among the normal animals, one dog with a pancreatic duct ligation excreted excessive amounts of fecal fat phosphorus while on the corn-oil fatty acid diet. No explanation for this is apparent.

Based on the premise that the presence of phospholipids in the feces indicates hydrolysis of fat, Pessoa *et al.* interpret their findings to indicate that hydrolysis of fats can occur in the absence of bile and that bile is necessary for the absorption of phospholipid. The smaller quantities of fecal phospholipid excreted by dogs with biliary fistulas ingesting tallow fat rather than corn oil is thought to be related to the fatty acid contents of these two fats. They cite the observations of K. S. Kim and A. C. Ivy (*Am. J. Physiol.* **171,** *302 (1952)*), that palmitic acid, a saturated fatty acid, stimulates less phospholipid excretion in normal rats than oleic acid, an unsaturated acid. Tallow fat consists chiefly of saturated fatty acids, whereas corn oil consists chiefly of unsaturated fatty acids.

The minimal fecal phospholipid excretion in the dogs with pancreatic duct ligations on the various diets is difficult to interpret. Does it mean that hydrolysis of fat is not occurring, or, perhaps, that what phospholipids are formed are readily absorbed in the absence of pancreatic secretion?

This study of Pessoa and co-workers emphasizes again the lack of specific information regarding the mechanism of fat digestion and absorption.

# NOTES

### Metabolic Rate in Diabetic Acidosis

Energy metabolism measured by oxygen consumption and basal metabolic rate (BMR) in patients with diabetic acidosis was found to be much higher than that of hospital patients without diabetes by P. Fisher and J. Kleinerman (*J. Clin. Invest.* **31,** *126 (1952)*). The subjects inhaled ambient air and expired into a Tissot spirometer. The expired air was analyzed for oxygen and carbon dioxide in the Haldane apparatus. In the 11 diabetic patients with severe acidosis the respiratory quotient (RQ) varied from 0.49 to 0.76 with a mean of 0.62. The oxygen consumption varied from 225 to 484 cc. per minute and the BMR from +3 to +77 per cent with a mean of +41.4 per cent (standard error 6.9). Two of the patients with diabetic acidosis were again studied after therapy had been instituted and it was found that their BMR's had dropped from +19 to +7 in one and from +25 to +3 in the other. There were 10 patients with diabetes so studied.

Seven hospital patients without diabetes were studied in a similar manner. Here the mean RQ was 0.79, the mean oxygen consumption 241 cc. (range 143 to 410 cc.), and the mean BMR −2.7 per cent (standard error 10.7; mean −41 to +37).

The authors concluded that there was a marked increase in the metabolic rate of the patients with diabetic acidosis. Hyperventilation was considered as a possible explanation. To test this hypothesis, a group of medical students and physicians hyperventilated, but they were unable to increase significantly their BMR's.

It seems clear from the figures presented that there was a marked increase in oxygen consumption and in BMR in the patients with diabetic acidosis. Whether hyperventilation can be excluded as a possible cause on the basis of the control studies with normal persons seems doubtful since it is difficult for normal individuals to hyperventilate to the extent accomplished by a diabetic with severe acidosis. The term BMR used in this paper seems somewhat inaccurate, since the way it was determined could hardly have been under standard conditions. The wide variation from the normal BMR of the patients without diabetes lends support to the propriety of questioning the method as measuring true BMR.

Interpretation of an increased oxygen consumption is certainly difficult in these patients. Surely it must depend at least in part upon the metabolic mixture. To define this metabolic mixture in these individuals, measurements of the nitrogen excretion would be necessary so that one could calculate the amount of oxygen required for the metabolism of nitrogen and the nonprotein RQ. Nitrogen studies of this kind were not included.

The possibility that there is an impaired synthesis of high energy phosphorus compounds in the absence of insulin (B. D. Polis, E. Polis, M. Kerrigan, and L. Jedeikin, *Arch. Biochem.* **23,** *505 (1949)*) suggested to Fisher and Kleinerman that the conversion of oxidative energy to work though energy-rich phosphate might be impaired in diabetic acidosis and "an acceleration of oxidative processes might be necessary to maintain normal cellular function." Whether this is the case or not would seem to depend upon the validity of the figures obtained upon repeated examination in the same individual, a comparison with a larger group of hospital patients without diabetes so that the wide variation could be controlled, and the calculation of the nonprotein RQ.

### Recent Nutrition Book

*Algal Culture. From Laboratory to Pilot Plant.* John S. Burlew, Editor. Carnegie Institution of Washington Publication 600, Washington, D. C., 1953. Pp. 325. Price: $1.25.

## CLINICAL NUTRITION BRIEFS

"The leading cause of mortality among diabetics is no longer severe acidosis and coma, but atherosclerosis and other degenerative lesions. This complicating disease develops early in diabetic patients, and progresses rapidly, affecting especially the coronary arteries and the arteries of the lower extremities." (*Nutrition Reviews* **11,** *292* (*1953*), Overweight and Obesity as a Medical Hazard.)

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"On the basis of work published to date, it appears that fructose ultimately may prove to be the sugar of choice in the parenteral nutrition of certain patients in whom glucose utilization is impaired. Diabetes mellitus and severe parenchymal liver disease are examples of conditions in which fructose administration sometimes may be preferable." (*Nutrition Reviews* **11,** *299* (*1953*), Fructose and Invert Sugar Versus Glucose.)

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"This condition has been termed 'relative anemia' by these authors. However, in view of the low oxygen saturation present in the blood of these patients and the necessity of their maintaining hemoglobin concentrations at abnormally high levels to prevent tissue anoxia, it differs in no significant way from the anemia of iron deficiency in normal children." (*Nutrition Reviews* **11,** *323* (*1953*), Iron Deficiency in Congenital Heart Disease.)

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"The progress in the fluoride problem during the past thirty-five years, beginning with an epidemiologic survey of an obnoxious public health problem, to epidemiologic surveys of a beneficial phase of the subject, to field testing of the agent and finally widespread adoption of water fluoridation as a public health measure is dramatically brought out in these papers." (*Nutrition Reviews* **11,** *324* (*1953*), Water Fluoridation as a Public Health Measure.)

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"It is inherent in the nature of situations in which survival itself becomes the goal that only minimal amounts of food and, frequently, of water, can be made available. Consequently, caloric balance cannot be maintained and the possibility of maintaining water balance is questionable. The principal problem in the design of a survival ration is thus reduced to furnishing nutrients which will minimize, if possible, the breakdown of body protein without increasing the obligatory urine volume." (*Nutrition Reviews* **11,** *327* (*1953*), Survival on Rations of Low Caloric Content.)

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"It would appear that gamma globulin breakdown is more rapid in cirrhotic than in normal subjects. This finding is of considerable interest in patients with liver disease, for, in view of the high incidence of hypergammaglobulinemia in patients with cirrhosis, increased metabolic decay as observed in these studies suggests a very high rate of gamma globulin production in order to maintain the high plasma levels observed." (*Nutrition Reviews* **11,** *329* (*1953*), Tagged Albumin and Globulin Metabolism in Cirrhosis.)

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"Folacin was administered to 20 premature infants in oral doses varying between 5 and 30 mg. and in parenteral doses of 15 to 30 mg. for variable periods of time. Five of these infants responded by a marked reduction in the excretion of hydroxyphenyl derivatives. Two of these infants responded when the folacin was given by intramuscular injection after failure of response during a period of oral administration. Those infants failing to respond to folacin responded in the expected manner to ascorbic acid." (*Nutrition Reviews* **11,** *332* (*1953*), Further Studies on Hydroxyphenyluria.)

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"One infant developed a severe microcytic hypochromic anemia, the other, convulsive seizures. All symptoms were corrected by administration of pyridoxine. These several reports leave no reasonable doubt that vitamin $B_6$ is essential in man as it is in experimental animals." (*Nutrition Reviews* **11,** *353* (*1953*), Supplement to Present Knowledge in Nutrition: Vitamin $B_6$.)